REMARKS

Reconsideration is requested.

Claim 11, 30 and 31 have been canceled, without prejudice. Claims 32-35 have been added and find support, for example, in the specification in the paragraph spanning pages 26-27 and the corresponding Example, and Examples 9 and 10. Moreover, claim 1 has been revised based on, for example, the disclosure of Example 6, Table 8, Example 10. No new matter has been added.

Claims 1, 3-7, 10, 14, 15, 17-24 and 27-29 and 32-35 are pending.

The Section 103 rejection of claims 30 and 31 over Bebbington in view of Brandt and Meyers (U.S. Patent No. 6,110,663) is moot in view of the above amendments.

The Section 103 rejections of claims 1, 3-7, 10, 11, 19-24 and 27 over Bebbington (U.S. Patent No. 5,891,693) "as evidenced by" Barsomian (U.S. Patent No. 5,238,821), in view of Brandt (U.S. Patent No. 6,395,484); and of claims 14, 15, 17 and 18 over Bebbington "as evidenced by" Barsomian, in view of Brandt and Hermentin (U.S. Patent No. 6,096,555), are believed to be obviated by the above amendments. Reconsideration and withdrawal of the rejections are requested in view of the above and the following comments.

The presently claimed invention provides a glutamine-auxotropic human cell transfected with a first exogenous DNA sequence comprising a DNA sequence encoding a sialylated protein and a DNA sequence encoding a selectable marker selected from the group consisting of DHFR, adenosine deaminase, asparagine synthetase, aspartate transcarbamylase, metallotionein-1, omithine decarboxylase, P-

glycoprotein, ribonucleotide reductase, thymidine kinase and xanthine-guanine phosporibosyl transferase, and (b) a second exogenous DNA sequence encoding a glutamine synthetase as a selectable marker, wherein the first exogenous DNA sequence and the second exogenous DNA sequence are located on different DNA constructs and the DNA constructs are vectors, and

wherein the rate of synthesis of the sialylated protein is elevated when the transfected cell is grown in a glutamine-free media as compared to a control cell not containing the second exogenous DNA sequence grown in media containing glutamine, and wherein the transfected cell produces a reduced concentration of ammonia in a glutamine-free media as compared to the concentration of ammonia the control cell produces in media containing glutamine.

The Examples of the specification demonstrate the elevated rate of synthesis of a sialylated protein when a transfected cell according to the claimed invention is grown in a glutamine-free media as compared to a control cell not containing the second exogenous DNA sequence which is grown in media containing glutamine. The Examples further demonstrate the reduced concentration of ammonia produced by a transfected cell according to the claimed invention in a glutamine-free media as compared to the concentration of ammonia a control cell not containing the second exogenous DNA sequence produces in media containing glutamine. The applicants have unexpectedly discovered that glutamine-auxotropic human cells are able to increase sialylation and/or N-glycan charge of a glycosylated protein expressed the cell if the cell is transfect with an exogenous DNA sequence encoding a glutamine

synthetase and grown or cultured in a glutamine-free media. The presently claimed cells reduce the concentration of ammonia in cell culture or media when grown in a glutamine-free media or culture which allows for a greater rate of protein synthesis and increased maximum product concentration (see for example, page 25, lines 3-7 of the specification) and increased degree of sialylation of the expressed glycosylated product (see for example, page 27, lines 2-5 of the specification).

The recited characteristics inherent to the claimed cells would not have been obvious from the combination of art cited by the Examiner. Specifically, Brandt is understood to exemplify culturing a human fibrosarcoma cell line (HT1080) transfected with a plasmid containing an antibiotic resistance gene (NEO), the DHFR gene and a DNA sequence encoding EPO. See columns 9 and 10 of Brandt. The transfected cell is cultured in DMEM containing serum and glutamine. Brandt indicates in Table 1 of the patent that HT1080 could be grown in a serum free media. The patent further states generally that a positive selection marker gene

> "can be any selection marker gene suitable for eukaryotic cells which upon expression leads to a selectable phenotype, e.g., antibiotic resistance, auxotrophy etc. The neomycin phosphotransferase gene is an especially preferred positive selection marker." See column 4, lines 46-51 of Brandt.

As noted above, Brandt exemplifies the "especially preferred positive selection marker." Brandt fails to specifically teach or suggest glutamine synthetase as a selectable marker. Brandt does not teach or suggest a glutamine-auxotropic human cell capable of being grown in glutamine-free media.

Brandt does not completely characterize the EPO produced in the Examples such as by demonstrating the quality of any isoforms produced. Brandt does not teach or suggest a elevated rate of synthesis of a sialylated protein when the transfected cells are grown in a glutamine-free media as compared to a control cell, as presently claimed. Brandt does not teach or suggest a reduction in ammonia concentration in the culture media as compared to non-transfected cells, as presently claimed.

Bebbington is understood to exemplify transfection of rodent cell lines (see column 6, lines 63-67) with an active glutamine synthase and a heterologous gene of interest.

The further cited Barsomian and Hermentin are understood to be cited to demonstrate that tPA is a sialylated glycoprotein and that sialylation is defined by N-charge and silylated proteins contain various glycoforms of N-glycans.

The combination of cited art however is not believed to have made the claimed invention obvious. Specifically, the there was no motivation or suggestion in the art to produce the claimed invention. Moreover, the claimed invention has been demonstrated to yield unexpected advantages which were no reasonably predictable from the combination of cited art. The presently claimed invention is more than a combination of known elements which yield a predictable result.

Withdrawal of the Section 103 rejections is requested.

The claims are submitted to be in condition for allowance and a Notice to that effect is requested. The Examiner is requested to contact the undersigned, prefer ably by telephone, in the event anything further is required in this regard.

Respectfully submitted,

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